because there is insufficient antecedent basis for the limitation "mutant cell" in the claim. This rejection is respectfully traversed.

Claim 1 recites "a mutant of a parent filamentous fungal cell." The "mutant cell" in claim 8 refers antecedently to the term "a mutant" of the phrase "a mutant of a parent filamentous fungal cell" in claim 1. However, to clarify the antecedent basis, the corresponding new claim 70 recites "a mutant cell of a parent filamentous fungal cell."

For the foregoing reason, Applicants submit that the rejection under 35 U.S.C. § 112, second paragraph, has been overcome and respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 1, 2, 8, 9, 13, and 22-24 under 35 U.S.C. § 112, First Paragraph

Claims 1, 2, 8, 9, 13, and 22-24 stand rejected under 35 U.S.C. § 112, first paragraph, "because the specification, while being enabling for a Fusarium fungal cell producing less cyclohexdepsipeptide and a method of producing a heterologous polypeptide using said cell, does not reasonably provide enablement for any filamentous fungal cell producing less cyclohexadepsipeptide." Specifically, the Office Action states:

> The relative skill of those in the art is low in making such cyclohexadepsipeptide-deficient cells in other filamentous fungi without being provided with the DNA sequences of those enzymes involved in the biosynthesis of cyclohexadepsipeptide in those particular cells. As making fungal cells with lowered levels of cyclohexdepsipeptide requires knowledge of the DNA sequences encoding enzymes involved in cyclohexadepsipeptide biosynthesis, there is unpredictability in making such cells other than in Fusarium, where the instant specification discloses a DNA sequence encoding a Fusarium cyclohexadepsipeptide synthetase. Additionally, the prior art of record does not indicate that those DNAs encoding enzymes involved in the biosynthesis of cyclohexadepsipeptide are known for other filamentous fungi besides Fusarium. Undue experimentation would be required to enable the full scope of the invention based upon the limited scope of the disclosure.

This rejection is respectfully traversed.

Applicants' contribution to the art is the discovery of producing secreted heterologous polypeptides using a mutant cell of a parent filamentous fungal host cell where the mutant cell is made deficient in its ability to produce cyclohexadepsipeptide compared to the parent filamentous fungal cell. The Office Action indicates that the specification is enabling for a Fusarium cell producing less cyclohexdepsipeptide and a method of producing a heterologous polypeptide using said cell, but is not enabling for other filamentous fungal cells. Applicants respectfully disagree.

Applicants have provided detailed instructions on how to produce mutant cells by deleting or

disrupting nucleic acid sequences encoding cyclohexadepsipeptide synthetases in filamentous fungal cells (see page 5, line 14 to page 8, line 3 and Examples 5 and 6). Based on the information provided by the Applicants in the specification and the knowledge available in the pertinent art, one skilled in the art can construct deletion vectors for transformation into any filamentous fungal cell, shown to produce cyclohexadepsipeptide, to disrupt or delete a gene involved in the biosynthesis of cyclohexadepsipeptide. For example, by selecting a highly conserved region of the *Fusarium* gene of SEQ ID NO: 1 relative to other similar genes in the prior art, a deletion vector can be prepared without knowledge of the corresponding gene sequence in another filamentous fungal cell. It is reasonably predictable that such a conserved region will be relatively homologous with similar genes from other filamentous fungi whether a *Fusarium* strain or not. Thus, a DNA fragment containing the conserved region interrupted with a selectable marker or a DNA fragment with a portion of the conserved region removed by digestion with a restriction enzyme can predictably then be used to replace the corresponding similar gene via homologous recombination in any filamentous fungal cell that produces cyclohexdepsipeptide. In fact, Herrmann *et al.* (*Molecular Plant-Microbe Interactions* 9: 226-232, 1996) have shown that an internal fragment of the *Fusarium scirpi esyn1* gene was useful in disrupting the *Fusarium avenaceum* enniatin synthetase gene without any knowledge of the nucleic acid sequence of the *Fusarium avenaceum* gene.

The Office Action also indicates that such genes have not been reported in other filamentous fungal cells besides *Fusarium*. Applicants respectfully point out that the production of cyclohexadepsipeptides have been reported by *Polyporus* (Deol *et al.*, 1978, *Aust. J. Chem.* 31: 1397-1399) and *Alternaria* (McKee *et al.*, 1997, *Journal of Natural Products* 60: 431-438), both filamentous fungi. While genes encoding cyclohexadepsipeptides have not yet been isolated from *Polyporus* and *Alternaria*, the need for isolation of the genes and delineation of the nucleic acid sequences is not necessary to disrupt or delete a gene involved in the biosynthesis of cyclohexadepsipeptide, as described above.

Applicants assert, therefore, that it is well within the skill of the art to make cyclohexadepsipeptide-deficient cells in filamentous fungi other than *Fusarium* using the nucleic acid sequences disclosed in the specification and the prior art without being provided with the DNA sequences encoding the enzymes involved in the biosynthesis of cyclohexadepsipeptide.

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112, first paragraph, have been overcome and respectfully request reconsideration and withdrawal of the rejections.

III. The Rejection of Claims 8, 9, 23, and 24 under 35 U.S.C. § 112, First Paragraph

Claims 8, 9, 23, and 24 stand rejected under 35 U.S.C. § 112, first paragraph, "because the specification, while being enabling for a filamentous fungal cell which produces less cyclohexadepsipeptide and which comprises a modification of the polynucleotide sequence of SEQ ID NO:1 and a method of producing polypeptides using said fungal cell, does not reasonably provide enablement for a filamentous fungal cell which produces less cyclohexadepsipeptide and which comprises a modification of at least one of the DNAs involved in the production of a cyclohexadepsipeptide and a method of producing polypeptides using said fungal cell." Specifically, the Office Action states:

> The breadth of the claims encompass a fungal cell comprising a modification of at least one of the DNAs involved in the production of cyclohexadepsipeptide. However, the instant specification only discloses the DNA of SEQ ID NO:1, which encodes a Fusarium cyclohexadepsipeptide synthetase. Insufficient examples and guidance are provided on other filamentous fungal DNAs involved in the production of cyclohexadepsipeptide. Besides DNA encoding Fusarium enniatin synthetase, the prior art of record does not teach any other DNAs encoding cyclohexadepsipeptide synthetase, enniatin synthetase or D-hydroxyisovalerate dehydrogenase. The skill of those in the art is low in making and using cyclohexadepsipeptide-deficient cells without being provided with the specific DNA sequence which encodes an enzymes involved in the biosynthesis of a cyclohexadepsipeptide. Absent such information, there is unpredictability in making fungal cells with reduced cyclohexadepsipeptide. Undue experimentation would be required to enable the full scope of the invention based upon the limited scope of the disclosure.

This rejection is respectfully traversed.

The Office Action asserts that the skill of those in the art is low in making and using cyclohexadepsipeptide-deficient cells without being provided with specific DNA sequences which encode enzymes involved in the biosynthesis of a cyclohexadepsipeptide (other than SEQ ID NO: 1) because the prior art of record does not teach any other DNAs encoding cyclohexadepsipeptide synthetase, enniatin synthetase or D-hydroxyisovalerate dehydrogenase. Applicants respectfully disagree.

As Applicants have demonstrated in Section II, it is well within the skill of the art to make cyclohexadepsipeptide-deficient cells in filamentous fungi other than *Fusarium* using the nucleic acid sequences disclosed in the specification and the prior art without being provided with the DNA sequences encoding the enzymes involved in the biosynthesis of cyclohexadepsipeptide. Moreover, Applicants have disclosed another source for a cyclohexadepsipeptide synthetase gene from *Fusarium scirpi* on page 1, lines 14-15, of the specification, and a source for the D-hydroxyisovalerate dehydrogenase gene from *Fusarium sambucinum* on page 7, lines 26-30, of the specification. One of

ordinary skill in the art can select highly conserved regions of these genes and construct deletion vectors without knowledge of the corresponding gene sequence of a similar cyclohexadepsipeptide biosynthetic gene in a different filamentous fungal cell shown to produce cyclohexadepsipeptide.

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112, first paragraph, have been overcome and respectfully request reconsideration and withdrawal of the rejections.

IV. The Rejection of Claims 1-3, 8, 9, 13, and 22-34 under 35 U.S.C. § 102

Claims 1-3, 8, 9, 13, and 22-34 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Herrmann *et al.* (*Molecular Plant-Microbe Interactions* 9: 226-232, 1996). The Office Action states:

> Herrmann et al. teach (page 227, "Disruption of the esyn1 gene," 1st and 2nd paragraphs) a Fusarium comprising a disruption of the enniatin synthetase gene by hygromycin B phosphotransferase, resulting in lowered levels of cyclohexadepsipeptides. Hygromycin B phosphotransferase is heterologously expressed in the Fusarium and used a selection marker. Claims 22-24 are therefore anticipated by Herrmann et al.
>
> With regard to claims 1-3, 8, 9, and 13, Herrmann et al. teach (page 231, "Enzyme purification and protein blotting") isolating the heterologous polypeptide hygromycin B phosphotransferase by preparation of a crude protein extract from cyclohexadepsipeptide-deficient Fusarium, which is that of claims 1-3, 8, 9 and 13.

This rejection is respectfully traversed.

Under the standard required for anticipation under 35 U.S.C. § 102, the cited prior art reference is required to disclose every element of the claimed invention. *Lewmar Marine Inc. v. Barient Inc.*, 3 USPQ2d 1766 (Fed. Cir. 1987).

Herrmann *et al.* disclose the effect of disruption of the enniatin synthetase gene on the virulence of *Fusarium avenaceum*. The disruption of the enniatin synthetase gene was accomplished with a gene disruption plasmid that also contained the hygromycin B phosphotransferase gene from *E. coli* as a selection marker.

However, Herrmann *et al.* do not disclose a method of producing a secreted heterologous polypeptide in a cyclohexadepsipeptide-deficient filamentous fungal cell, as claimed herein. Applicants' instant invention is directed to the production of secreted heterologous polypeptides, while Herrmann *et al.* disclose expression of hygromycin B phosphotransferase which is not a secreted enzyme.

For the foregoing reason, Applicants submit that the rejections under 35 U.S.C. § 102 have been overcome and respectfully request reconsideration and withdrawal of the rejections.

IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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